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VARIATIONS IN SKIN SURFACE LIPID COMPOSITION AND SEBUM EXCRETION RATE WITH DIFFERENT SAMPLING TECHNIQUES II

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Summary.—The technique of Sellotape stripping was used to investigate the role of the stratum corneum in modifying sebum excretion rate and skin surface lipid composition.

All experiments were performed on the forehead. The stratum corneum influences the rate of sebum excretion as Sellotape stripping produced a significant increase in sebum excretion rate, but this effect was abolished by systemic antibiotics.

Skin surface lipid composition, when sampled by absorbent papers, was not influenced by Sellotape stripping, but when sampled by an ether-soaked sponge after Sellotape stripping, had a lessened epidermal lipid contribution. Thus the stratum corneum modifies skin surface lipid composition when sampled by an organic solvent.

Absorbent papers may be preferable to other sampling techniques on the forehead in the investigation of the role of sebaceous biochemical factors in the pathogenesis of acne vulgaris.

THE skin surface lipid composition of the forehead varies considerably with the method of collection (Cunliffe *et al.*, 1971). When sampled by an organic solvent the surface lipid has a composition which reflects a greater epidermal contribution than does surface lipid collected by absorbent papers which sample lipid with a greater contribution from the sebaceous glands (Cunliffe *et al.*, 1971). In the present study we have investigated the role of the outermost part of the epidermis in modifying skin surface lipid composition.

MATERIAL AND METHODS

We investigated 41 patients, 23 females and 18 males, all of whom had varying degrees of acne vulgaris. Their ages ranged from 14 to 31 years. Eighteen patients had been taking either tetracycline 250 mg, trimethoprim-sulphamethoxazole (80 mg trimethoprim and 400 mg sulphamethoxazole) or Clindamycin 150 mg daily for at least 1 month prior to the investigation, whereas none of the other subjects was receiving any topical or systemic therapy. The patients washed their hair on the evening preceding the collection, but did not wash the face on the morning of the test.

In 21 patients, including 8 who were receiving antibiotics, absorbent papers were applied to both sides of the forehead until a follicular pattern was obtained. At this stage of the experiment one side of the forehead had the outermost part of its stratum corneum removed by using 1·5 in. wide transparent adhesive tape (Sellotape). The tape was pressed firmly on to the side and then removed, taking with it keratinocytes and

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bacteria. The stripping procedure was repeated 25 times, and the surface lipid was then collected from both sides of the forehead for 3 hours, using absorbent papers.

In another group of 20 patients, 10 taking antibiotics, a follicular pattern was again obtained on the forehead, and one side was likewise stripped 25 times with Sellotape. On the unstripped side sebum was collected by absorbent papers for 3 hours, but during this time period the Sellotape-stripped side was left exposed to the atmosphere. At the end of 3 hours the surface lipid was collected from the unstripped side, using 10 firm strokes with an Analar ether-soaked polyurethane sponge.

TABLE I.—*Differences in Skin Surface Lipid Composition and Sebum Excretion Rate between the Unstripped and Stripped Sides (+ = Stripped, > Unstripped; — = Unstripped, > Stripped). Skin Surface Lipid Was Collected on Each Side by Absorbent Papers. No Subject Was Taking Antibiotics*

Subject	Sebum excretion rate (μg/cm^2/min)	Squalene (%)	Wax ester (%)	Triglyceride (%)	Free fatty acid (%)	Cholesterol (%)
1	0·00	−2·0	−3·2	−9·0	+14·1	+0·2
2	−0·12	+1·6	−3·1	−5·3	+6·9	−0·1
3	−0·07	+3·9	+0·4	−10·2	+6·2	−0·3
4	−0·38	+0·1	−1·4	−0·8	+2·1	0·0
5	+0·02	+0·4	+2·5	+4·3	−6·6	−0·5
6	−0·34	+0·2	−0·2	−5·0	−4·7	−0·1
7	−0·14	+1·6	−0·8	−1·5	+0·6	0·0
8	+0·08	−1·5	−3·2	+10·2	−5·1	0·0
9	+0·06	+2·8	−0·7	+5·3	−7·3	−0·1
10	−0·37	−3·4	−5·8	+0·5	+8·2	0·0
11	+0·01	0·0	+1·3	−4·4	+3·4	0·0
12	−0·22	−3·1	+0·5	−19·3	+21·8	0·0
13	−0·14	+0·1	+4·0	−6·9	+2·9	0·0
A.M.	−0·13	+0·05	−0·71	−3·24	+3·99	−0·07
S.E.M.	0·05	0·08	0·74	2·11	2·25	0·04
t	2·85	0·08	−0·96	−1·53	1·77	−1·75
2p	0·02	0·90	0·30	0·1	0·1	0·1

TABLE II.—*Differences in Skin Surface Lipid Composition and Sebum Excretion Rate between the Unstripped and Stripped Side of the Forehead in* 8 *Subjects Receiving Antibiotics (+ = Stripped, > Unstripped; — = Unstripped, > Stripped). Skin Surface Lipid Was Collected on Each Side by Absorbent Papers*

Subject	Sebum excretion rate (μg/cm^2/min)	Squalene (%)	Wax ester (%)	Triglyceride (%)	Free fatty acid (%)	Cholesterol (%)
1	+0·32	−0·2	+1·9	−2·0	+0·2	+1·0
2	+0·20	+0·5	−2·4	−3·6	+6·8	−0·5
3	+0·18	−1·2	−0·6	+0·9	+0·1	−0·2
4	−0·59	−3·7	+3·6	+1·8	−1·5	−0·1
5	0·58	+1·2	+2·3	−3·1	−0·4	+0·1
6	−0·13	+2·2	+12·2	−18·5	+3·8	+0·2
7	−0·21	+2·8	+17·8	−24·0	+3·6	−0·1
8	+0·09	+1·8	−1·8	+1·7	−1·5	−0·1
A.M.	−0·09	+0·42	4·12	−5·85	+1·39	0·03
S.E.M.	0·12	0·75	2·54	1·68	1·06	0·15
t	−0·72	0·56	1·62	−1·68	1·31	0·20
2p	0·4	0·5	0·1	0·1	0·2	0·8

The samples from the polyurethane sponge and paper were extracted, using 3 aliquots of 25 ml Analar ether. All samples were then immediately evaporated, using a rotary evaporator (Büchi) (Cotterill *et al.*, 1971a). The samples were flushed with nitrogen after weighing and then stored at — 20°C until the following day, when semiquantitative biochemical analysis was performed (Cotterill *et al.*, 1971a).

RESULTS

Table I shows the difference in sebum excretion rate in patients in whom the effect of Sellotape stripping on samples collected by papers alone was investigated.

TABLE III.—*Differences in Skin Surface Lipid Composition when Data Analysed After Collection by an Ether-soaked Sponge from the Sellotape-stripped Side of the Forehead were Subtracted from Data Obtained After Collection by Absorbent Papers from the Unstripped Side in* 10 *Subjects not Receiving Antibiotics* (+ = *Stripped,* < *Unstripped;* — = *Unstripped,* < *Stripped*)

Subject	Squalene (%)	Wax ester (%)	Triglyceride (%)	Free fatty acid (%)	Cholesterol (%)
1	+5·1	+3·1	+9·2	−17·3	−0·1
2	+1·8	−0·4	+3·4	+2·1	−0·1
3	+2·7	+2·9	+11·8	−17·1	−0·1
4	+0·7	−1·4	+12·7	−12·0	0·0
5	−3·3	−10·0	+8·5	+5·0	−0·2
6	+0·7	−1·4	+10·6	−8·7	−1·1
7	+3·4	+3·1	+11·3	−16·0	−1·9
8	+1·9	+4·6	−4·8	−1·5	−0·3
9	−4·2	+5·6	+5·1	−6·2	−0·4
10	+7·7	−8·4	+11·5	−12·1	+1·2
A.M.	+1·65	−0·23	+7·25	−8·38	−0·30
S.E.M.	1·12	1·68	2·01	2·54	0·25
t	1·47	−0·14	3·60	−3·30	−1·19
2p	0·1	0·8	0·01	0·01	0·2

TABLE IV.—*Differences in Skin Surface Lipid Composition when Data Analysed After Collection by an Ether-soaked Sponge from the Sellotape-stripped Side of the Forehead Subtracted from Data Obtained After Collection by Absorbent Papers from the Unstripped Side in* 10 *Subjects Taking Antibiotics* (+ = *Stripped,* < *Unstripped;* — = *Unstripped,* < *Stripped*).

Subject	Squalene (%)	Wax ester (%)	Triglyceride (%)	Free fatty acid (%)	Cholesterol (%)
1	+1·1	+0·3	−0·2	−0·6	−0·7
2	+2·8	+1·4	+7·1	−9·7	−1·7
3	+1·6	+9·0	+14·5	−24·7	−0·4
4	−0·2	+1·8	+6·4	−7·6	−0·5
5	−1·1	−10·6	+12·0	−0·1	−0·1
6	+1·4	−4·9	+7·3	−2·8	−1·0
7	−1·2	−0·7	+10·5	−5·6	0·0
8	+3·2	−2·6	+13·5	−16·9	0·8
9	+0·7	+2·3	+6·6	−3·8	−0·5
10	−1·2	−3·7	+2·4	−3·5	−0·1
A.M.	+0·71	−0·77	+8·01	−7·53	−0·4
S.E.M.	0·51	1·64	1·49	2·46	0·21
t	1·39	−0·47	5·38	−3·06	−1·95
2p	0·1	0·6	0·001	0·02	0·1

When the subjects were not receiving antibiotics, the sebum excretion rate was significantly greater on the Sellotape-stripped side ($P < 0{\cdot}02$), but this difference was abolished in the antibiotic-treated subjects (Table II). In these subjects Sellotape stripping produced no significant changes in the percentages of squalene, wax ester, triglycerides, free fatty acids and cholesterol in either the antibiotic or non-antibiotic groups (Tables I, II).

Certain differences were found in the second group of 20 subjects in whom the surface lipid was collected by absorbent papers and compared to the lipid collected by sponging from the side which had been Sellotape-stripped (Tables III, IV). There were significantly greater percentages of triglyceride and significantly less free fatty acids in the paper samples. There were no significant differences in squalene, wax ester and cholesterol between the 2 sides, in either the antibiotic or non-antibiotic groups, and the probabilities are shown in Tables III, IV.

DISCUSSION

We have previously shown, using absorbent papers that there are no significant differences in sebum excretion rate (Cunliffe and Shuster, 1969) or skin surface lipid composition (Cotterill *et al.*, 1971a) between the right and left side of the forehead.

We used Sellotape stripping to assess the effect of the stratum corneum on sebum excretion rate and skin surface lipid composition. We accept that Sellotape stripping is perhaps not the ideal way of removing stratum corneum, but we were anxious not to use cyanoacrylate adhesive (Marks and Dawber, 1971) near the eyes. Furthermore, we do not know the effect of temperature on skin surface lipid composition; an increase of temperature is employed by Turek (1971) in his technique for the removal of stratum corneum. We have shown that Sellotape stripping produces an increase in sebum excretion rate, an effect which is abolished by antibiotics. In the acne patients not receiving antibiotics it is possible that Sellotape stripping will relieve some of the obstruction to sebum outflow by removal of the hyperkeratotic plugs which are characteristic of this condition. There is evidence in rabbits that sebaceous lipids and in particular free fatty acids may produce follicular hyperkeratosis (Kligman and Goldman, 1968), and those broad spectrum antibiotics which are effective in acne are known to reduce the free fatty acid composition of skin surface lipid (Beveridge and Powell, 1969; Cotterill *et al.*, 1971b). Thus long term treatment with antibiotics may be associated with less marked follicular plugging so that Sellotape stripping would, as we have found, have no effect on sebum excretion rate. It follows that long-term effective antibiotic treatment should perhaps increase sebum excretion rate, but there is no evidence that this in fact happens. However, our observations are in keeping with the idea that the cutaneous bacterial flora in acne subjects may lead directly or indirectly to follicular obstruction. An alternative explanation for the increased sebum excretion rate in patients not taking antibiotics could be an effect of the stripping on sebum production. Repeated Sellotape stripping is known to increase epidermal mitosis (Pinkus, 1952), but the slow turnover of sebaceous cells (Epstein and Epstein, 1966) and the failure to find any difference in the antibiotic treated group makes this explanation highly unlikely.

The stripping procedure had no significant effect on skin surface lipid composition sampled by absorbent papers, and thus the composition of surface lipid sampled by this method is not influenced by the presence of the outermost layer of the skin. This finding lends support to our contention that absorbent papers sample lipid more closely approaching pure sebum in composition than do other sampling techniques. We realize that there may be selective loss of lipids for subsequent analysis when samples are taken by absorbent papers, but precise data on this possibility is not available, and this problem is under active investigation.

In the present studies sponging after Sellotape stripping sampled significantly more free fatty acids and significantly less triglyceride than the absorbent papers. However, there were no significant differences in squalene, wax ester and cholesterol. This is in striking contrast to our previous work on sponged unstripped skin (Cunliffe *et al.*, 1971), where we have shown that samples of skin surface lipid obtained by the sponge technique as compared to the paper technique contained not only significantly less triglyceride and more free fatty acid but also significantly less wax ester and squalene and significantly more cholesterol. We interpreted these findings to mean that the sebum collected by absorbent papers approximates more to the composition of pure sebum than that collected by sponging, which has a greater epidermal contribution. It is clear that samples obtained by sponging after Sellotape stripping eliminate the differences we have previously found in wax ester, squalene and cholesterol and sample skin surface lipid, with a lesser epidermal contribution than that from unstripped skin.

We conclude that the stratum corneum influences the rate of sebum excretion in untreated acne subjects, probably by modifying resistance to sebum outflow, and that absorbent papers may be preferable to other sampling techniques on the forehead in the investigation of the role of sebaceous biochemical factors in the pathogenesis of acne vulgaris.

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